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| Curriculum

Helping students see bacteria in 3D: cellular models increase student learning about cell size and diffusion

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ABSTRACT In the microbial world, cell size and shape impact physiology, but students struggle to visualize spatial relationships between cells and macromolecules. In prokaryotic cells, cell size is limited by reliance on diffusion for nutrient uptake and the transport of nutrients within the cell. Cells must also meet a minimum size threshold to accommodate essential cellular components such as ribosomes and DNA. Using 3D printing allows for the creation of custom models that can be influential teaching tools in the biology classroom. This lesson uses 3D cell models to teach students enrolled in an introductory microbiology course about bacterial cell size and the biological importance of surface-area-to-volume ratio. During the lesson, students interact with 3D cell models and discuss a series of questions in small groups. Student learning was assessed using quantitative and qualitative student response data collected pre- and post-lesson. Student achievement of learning objectives, and their confidence in their knowledge of these concepts, improved post-lesson, and these gains were statistically significant. Our findings suggest that interacting with 3D-printed cell models improves student understanding about bacterial cell size and diffusion.

KEYWORDS cell models, 3D printing, cell size, cell shape, cell physiology, microbiology education

Cell size and shape impact nutrient flux across the cell membrane and diffusion of molecules within the cytoplasm, ultimately influencing rates of metabolism and growth (1). In eukaryotes, organelles and a complex cytoskeleton are used to compartmentalize reactions and optimize intracellular organization and transport, allowing eukaryotic cells to reach larger sizes (2, 3). Prokaryotic cells—that is, cells without a membrane-bound nucleus including both bacteria and archaea—typically lack organelles, so they rely on diffusion for uptake and transport reactions. Thus, the physiology and metabolism of prokaryotic cells are fundamentally controlled by membrane surface area and the distance required for molecules to diffuse within the cytoplasm, limiting their size (1, 4). Prokaryotic cells must also meet a minimum size threshold to accommodate essential cellular components such as ribosomes and DNA (1). There are some bacteria that defy these norms and achieve sizes significantly larger than the typical prokaryotic or eukaryotic cell. For example, a *Thiomargarita namibiensis* cell is generally about 100–300 µm in diameter, a feat it achieves by possessing a large central storage vacuole that allows it to maintain a thin layer of cytoplasm adjacent to the cell membrane (5).

Despite the physiological importance of cell size and structure, many biology textbooks fail to mention the impact of cell size on diffusion (6) and display misleading images of cellular components such as mitochondria and chloroplasts (6, 7). Figures comparing the components of prokaryotic and eukaryotic cells often display them side

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by side, with each cell shown as approximately the same size, potentially leading to student misunderstandings about the relative sizes of cells.

One way to overcome some of these misunderstandings is to have students interact with 3D models that provide an opportunity to actively engage with microbiological concepts and visualize microscopic cells and macromolecules. Physical models have been previously used to illustrate the structure and function of cellular macromolecules and to demonstrate the molecular basis of laboratory techniques such as PCR as described in many published lessons (8–14). In recent years, 3D printing has risen in popularity and become widely available at universities through the development of "Makerspaces" (15). Using 3D printing provides a unique teaching opportunity allowing for the creation of custom models, a strategy that has been previously implemented to provide students with physical representations of macromolecules, enzymes, and operons (16–19).

There is evidence that models improve student understanding of cell biology concepts. One study compared student performance between groups with access to 3D models to those without access in a cell biology laboratory course. The students with access to the models performed better on interview questions about a protein not discussed in class compared to their peers without access to the models; however, the students with access to models did not achieve higher grades in the course overall (20). In addition to potentially improving student learning, employing 3D models can create a more inclusive classroom, which accommodates the needs of visually impaired and blind students (21). In fact, 3D printing has been successfully used to tactilely present visual biochemistry and chemistry data such as images, plots, and gels (22, 23).

Despite the potential benefits of integrating 3D-printed models into the biology classroom, few studies have attempted to quantify student learning using pre- and post-assessments (24). Here, we describe a lesson which seeks to correct student misunderstandings about cell size by using to-scale, 3D-printed cell models to illustrate the relative size of bacterial cells and to guide students in considering the biological significance of cell size and shape. Furthermore, we present data from a pre- and post-lesson assessment that suggests completion of our lesson, including interacting with the 3D-printed models, results in a significant increase in students' knowledge of and confidence in the lesson learning objectives.

Intended audience

This lesson was designed to be taught in the discussion sections of an introductory microbiology lecture course. Most students are sophomores who have completed one year of introductory biology and chemistry. A summary of the class standing and declared majors of students enrolled during the Spring 2022 semester can be found in Table 1. Each discussion section has about 20 students enrolled, but this lesson could be taught in a smaller or larger class. It could also be implemented during a lecture or lab section of a course.

Learning time

This lesson is intended to be taught over one 50-min class period. Students are also asked to take a short pre- and post-lesson assessment outside of class, each of which can be completed in approximately 5–10 min.

Prerequisite student knowledge

Prior to the lesson, students were introduced to the concepts of bacterial cell size, diffusion, surface-area-to-volume ratio, and active transport in lecture. Students should generally understand biology concepts typically covered in high school and introductory biology courses including diffusion, active transport, and the functions of cellular components such as ribosomes and DNA. However, this lesson can easily be modified for majors or non-majors with varying levels of prior knowledge.

TABLE 1 Class standing and declared major of students enrolled in the course[a]

Class standing	Number of students	Major	Number of students
First Year	4	Biology & Allied Health	120
Sophomore	93	Food Science	19
Junior	61	Animal Science	31
Senior	38	Other	29
Graduate	3		

[a]Class standing and declared majors for students enrolled in the course during the Spring 2022 semester. Other majors include Plant Sciences, Environmental Sciences, Engineering, and others.

Learning goals

The content of this lesson relates to the following learning goals outlined in the ASM Curriculum Guidelines for Undergraduate Microbiology (25):

- Scientific thinking: Use mathematical reasoning and graphing skills to solve problems in microbiology
- Metabolic pathways: The growth of microorganisms can be controlled by physical, chemical, mechanical, or biological means

Learning objectives

After completing the lesson, students should be able to:

- LO-1: Calculate surface-area-to-volume ratios of cells
- LO-2: Compare the relative sizes of eukaryotic and bacterial cells
- LO-3: Explain how surface-area-to-volume ratio affects biological processes

PROCEDURE

Materials

Materials for this lesson include a lesson plan (Supplemental Material S1. Lesson Plan), student worksheet (Supplemental Material S2. Lesson Worksheet), and a pre- and post-lesson assessment (Supplemental Material S3. Lesson Assessment). An answer key for the student worksheet can be provided on request by emailing the corresponding author. This lesson also requires two different sets of models. For Part I of the lesson, each of the following models is required for each small group: a 3D-printed model of *Escherichia coli* and *Staphylococcus aureus*, a nucleoid constructed out of monofilament fishing line, and a packet of 3 mm beads representing ribosomes (Fig. 1A). Part II requires one set of 3D-printed models for the class including an *E. coli*, an *S. aureus*, a human osteoblast, a human intestinal epithelial cell, and the giant bacterium *T. namibiensis* (Fig. 1B). The colors of the bacterial models in Part II were selected to illustrate common staining techniques. *E. coli* is Gram-negative (pink), *S. aureus* is Gram-positive (purple), and *T. namibiensis* is green to reflect staining of the cytoplasm with fluorescein isothiocyanate. The models for Part I and Part II are at different scales due to the large size of *T. namibiensis* and the printer bed size constraints of standard 3D printers, but within each set, all the models are to-scale. The nucleoids are constructed from about 150 m of fishing line and are approximately to-scale in terms of chromosome length, but the 0.35-mm wide filament is roughly 1.75 times the width of to-scale DNA. For printable 3D model design files, additional information about the models, and tips for 3D printing see Supplemental Material S4. 3D Printing Instructions and Description of Models.

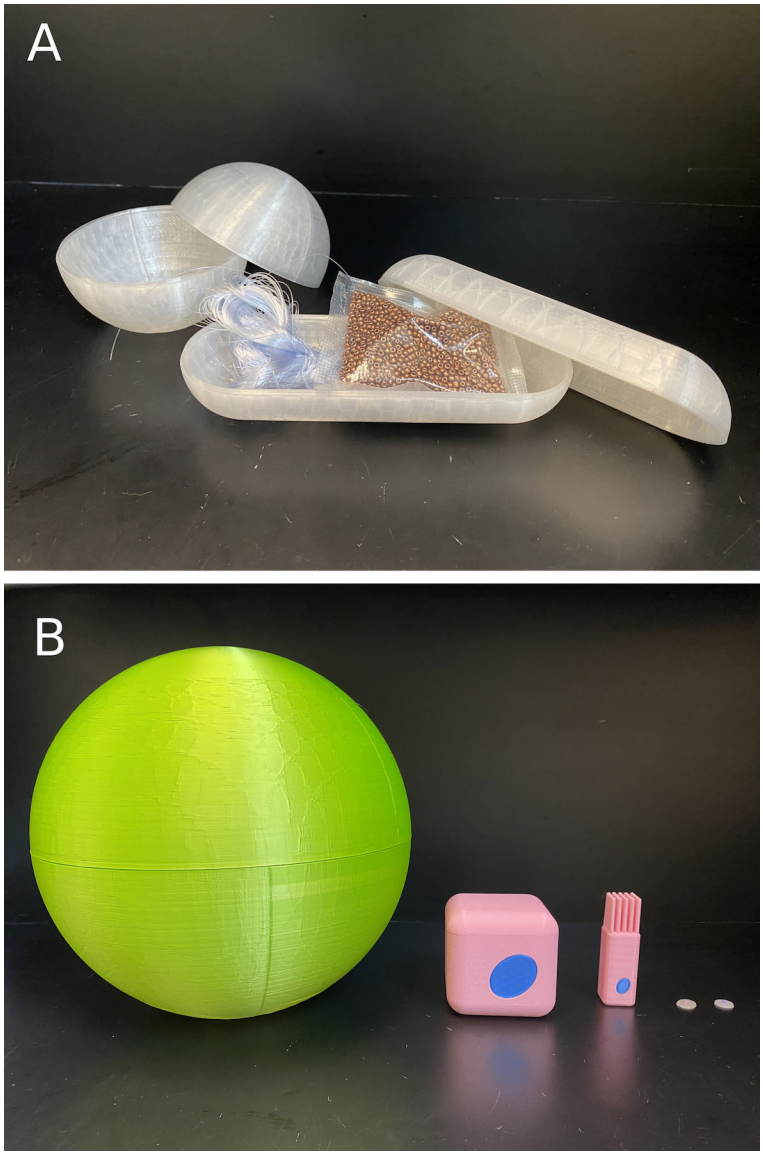

FIG 1 Models for Part I and Part II of the lesson. (A) Models for Part I of the lesson include *E. coli* (rod) and *S. aureus* (coccus) cells, a nucleoid (blue spindle), and a packet of ribosomes (3 mm brown beads). Students can place cellular components inside of the interactive cell models. (B) Models for Part II include (from left to right) *T. namibiensis*, a human osteoblast, a human intestinal epithelial cell, *E. coli*, and *S. aureus*. These models show the size differences between various cell types.

Student instructions

The student instructions for the activity are provided on a worksheet to be handed out during class (Supplemental Material S2. Lesson Worksheet). We recommend printing the worksheet in color if possible. Alternatively, instructors could share the color version of the worksheet on a learning management system or provide printed color copies of the images on page 2 and 5 for groups of students to share. Students are also asked to complete a pre- and post-lesson assessment (Supplemental Material S3. Lesson Assessment). These can be shared with students using a learning management system or on paper.

Faculty instructions

Prior to class, the instructor asks students to complete a low-stakes pre-lesson assessment graded for completion. The assessment evaluates students' knowledge about cell size and diffusion and gives students an opportunity to estimate the relative size of cells before seeing 3D models that demonstrate their actual relative sizes. Students are not given answers to this assessment before class.

In class, the instructor begins by explaining that the class session will involve completing a lesson that uses 3D-printed cell models to explore the size of cells and their components and the biological impacts of surface-area-to-volume ratio. Students are organized into groups of three to five people and instructed to work on Part I using the models provided. In the worksheet, students are prompted to estimate and then calculate the number of ribosomes that could fit in each cell model (Supplemental Material S2. Lesson Worksheet—Part I, Questions 1–2) and answer two discussion questions that ask them to critically consider the reasons that cells need many ribosomes (Supplemental Material S2. Lesson Worksheet—Part I, Question 3). After the students complete Part I, the instructor asks for volunteers to share their answers to each question with the class. We encourage instructors to use equitable practices that encourage all students to participate such as waiting for multiple hands to be raised before calling on a student or assigning a reporter for each group (26).

Students then continue to work with their group to complete Part II. Students are asked to estimate which cell shape has a larger surface-area-to-volume ratio and calculate surface areas and surface-area-to-volume ratios for each of the cells from Part I (Supplemental Material S2. Lesson Worksheet—Part II, Questions 1–3). A set of class models for Part II (*E. coli*, *S. aureus*, human osteoblast, human intestinal epithelial cell, and *T. namibiensis*) are placed at the front of the room. The instructor prompts groups to take turns coming to the front of the room to examine the models which correspond to a table in the worksheet. Based on surface-area-to-volume ratios, students are asked to consider which cell types shown in the table will experience the most efficient diffusion of nutrients across the membrane (Supplemental Material S2. Lesson Worksheet—Part II, Question 4). After completing the table, students are asked to further consider the giant bacterium *T. namibiensis*. Despite its reliance on diffusion for nutrient uptake and transport, it achieves sizes that are much larger than the typical bacterium or human cells. The worksheet provides images of the internal structure of *T. namibiensis*, and students evaluate the impact of the large central vacuole on surface-area-to-volume ratio (Supplemental Material S2. Lesson Worksheet—Part II, Question 5). Finally, students are asked to consider aspects they could alter in a bioengineered cell to optimize diffusion (Supplemental Material S2. Lesson Worksheet—Part II, Question 6). Once all the groups have completed the activity, the instructor asks student volunteers to share their answers with the class. A lesson plan outlining the approximate timeline for the in-class activity is provided (Supplemental Material S1. Lesson Plan). As homework, students are asked to complete a low-stakes post-lesson assessment, graded based on completion, to evaluate knowledge gains (Supplemental Material S3. Lesson Assessment—Q1-5).

Suggestions for determining student learning

A pre- and post-lesson assessment was used to assess student learning (Supplemental Material S3. Lesson Assessment—Q1-5). The maximum score possible on the assessment was five. Furthermore, we encourage instructors to assess students on the concepts covered in this lesson on future quizzes or exams. Questions from our pre- and post-lesson assessment could be repurposed as exam or quiz questions. In our class, a series of true or false questions related to the concepts from the lesson were included on the midterm exam (Supplemental Material S3. Lesson Assessment—MQ1). We also evaluated student perceptions about the lesson and their learning in the pre- and post-lesson assessment (Supplemental Material S3. Lesson Assessment—SQ1-6). Student responses to open-ended questions were independently coded by three coders. Responses were

coded as positive (providing only positive comments), negative (providing only negative comments), or mixed (providing a combination of positive and negative comments). Responses including both negative and positive comments were coded as mixed regardless of the number of negative or positive comments. In cases of disagreement in the codes assigned between the coders (about 7% of the student statements), the consensus of two out of three coders was used.

Safety issues

This lesson and study were evaluated by the Cornell Institutional Review Board (protocol 2113010807) and determined to be exempt from institutional review. There are no safety issues associated with this lesson. Students provided informed consent via an online form for the use of their data in this study.

DISCUSSION

Field testing

The lesson was taught during the Spring 2022 semester in five discussion sections. Data from the pre- and post-lesson assessment were collected in these five discussion sections and one additional section that was canceled due to a fire alarm going off in the building at the start of class. A previous version of the lesson that used household objects such as jars to represent cells was taught for several semesters prior to being modified to instead use the 3D-printed models. Analysis of the student assessment data, including statistical tests and plotting, was conducted using R including the packages tidyverse and ggplot2 (27, 28).

Evidence of student learning

Based on analysis of the pre- and post-lesson assessment, we observed that students' knowledge of the learning objectives significantly increased after completing the lesson. Students achieved higher scores on a post-lesson quiz (Supplemental Material S3. Lesson Assessment—Q1-5) assessing their knowledge of the learning objectives, and this effect was statistically significant (Fig. 2, $n = 100$, $t(99) = 8.62$, $P < 0.001$). Students' scores increased by an average of 22.4% (1.12 points of 5 points total) from 2.30 (SD = 0.99) to 3.42 (SD = 1.10) out of 5. Overall, 76% of students' scores increased on the post-lesson assessment, with only 15% of students scoring lower and 9% experiencing no score change relative to the pre-lesson assessment (Fig. 3). Student performance improved on questions corresponding to all three of the learning objectives (Supplemental Material S5. Supplemental Figures—Fig. S2). These differences were statistically significant for all assessment questions (Table 2).

One class section was disrupted due to a fire alarm, so these students were unable to interact with the 3D models. However, they were introduced to the concepts of bacterial cell size, diffusion, surface-area-to-volume ratio, and active transport during a previous lecture. This unplanned situation provides an opportunity to assess the role of our 3D models in improving student learning. Students who completed the lesson experienced more improvement between the pre- and post-lesson assessments ($M = 1.12$, SD = 1.30, $n = 100$) than their peers whose class was canceled ($M = -0.047$, SD = 1.24, $n = 17$), and this difference was statistically significant [$t(115) = 3.57$, $P = 0.002$]. Although these data represent a small, non-random sample, which is subject to potentially confounding variables, it suggests that the lesson, including interaction with the 3D models, promotes greater student learning compared to only experiencing the lecture covering topics from the lesson (Supplemental Material S5. Supplemental Figures—Fig. S1). This conclusion is in general agreement with prior work that shows active learning strategies increase students learning relative to lecturing alone (29).

Students were also assessed on concepts from the lesson via a series of true or false questions included on the midterm exam that was administered about a month after the lesson (Supplemental Material S3. Lesson Assessment—MQ1). Students who completed

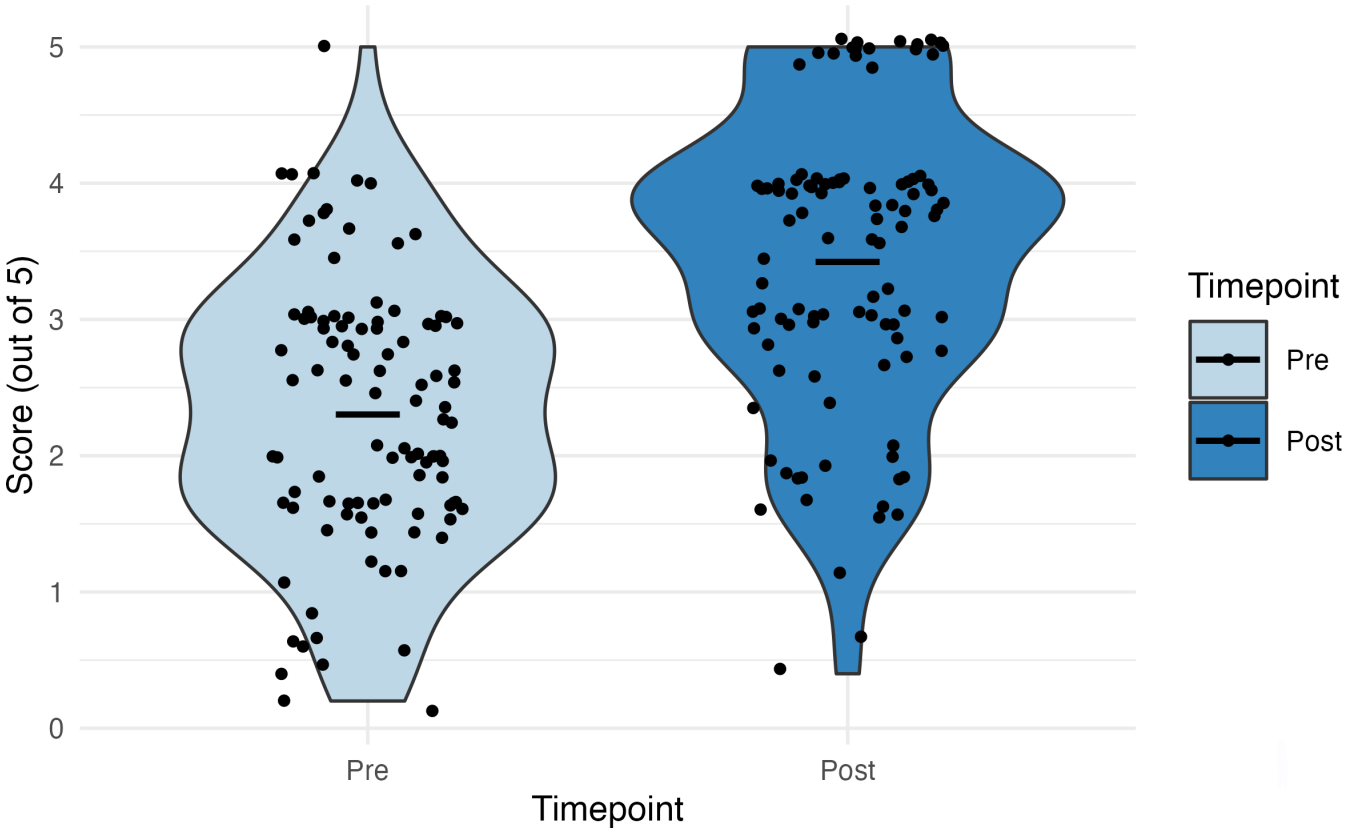

FIG 2 Student scores on a low-stakes quiz before and after completing the lesson Student scores on the pre- and post-lesson assessment indicate significant improvement. The average score is represented by a horizontal bar. Students' scores increased by an average of 1.12 points after completing the lesson (SD = 1.30). This observed increase was statistically significant based on a paired samples *t*-test ($n = 100$, $P < 0.001$). The effect size was large, with a Cohn's *d* value of 0.86.

the lesson performed slightly better on these questions on average scoring 3.05 ($n = 98$, SD = 0.83) compared to 2.85 ($n = 84$, SD = 0.74) out of 4, but this difference was not statistically significant at a 95% confidence level [$t(180) = 1.77$, $P = 0.08$]. Using a true or false format enables students to answer multiple questions more quickly, thereby allowing assessment of a broader range of content in a short time (30). However, the students have a 50% chance of guessing the correct answer. This high probability of students guessing correctly may, in part, explain the lack of difference in performance on the midterm questions between students who completed and did not complete the lesson. Although the difference in overall score on the midterm questions was not significant, a significantly greater number of students who completed the lesson answered a question about the quantity of ribosomes in a bacterial cell correctly on the midterm exam compared to their peers who did not complete the lesson (Supplemental Material S3. Lesson Assessment—MQ1B, $P = 0.01$). This result suggests that students who participated in the lesson may have obtained and retained a greater understanding of the relative size and quantity of ribosomes found in a typical bacterial cell. A summary of the student performance data from the midterm assessment questions can be found in Supplemental Material S5. Supplemental Figures—Table S1.

The overall increase in student performance after completing the lesson was accompanied by a significant improvement in student confidence in their knowledge of these concepts. After completing the lesson, students indicated significantly higher levels of confidence across all three learning objectives (Supplemental Material S3. Lesson Assessment—SQ1, $n = 100$, $P < 0.001$, Fig. 4). A summary of the changes in student confidence and the corresponding statistics can be found in Supplemental

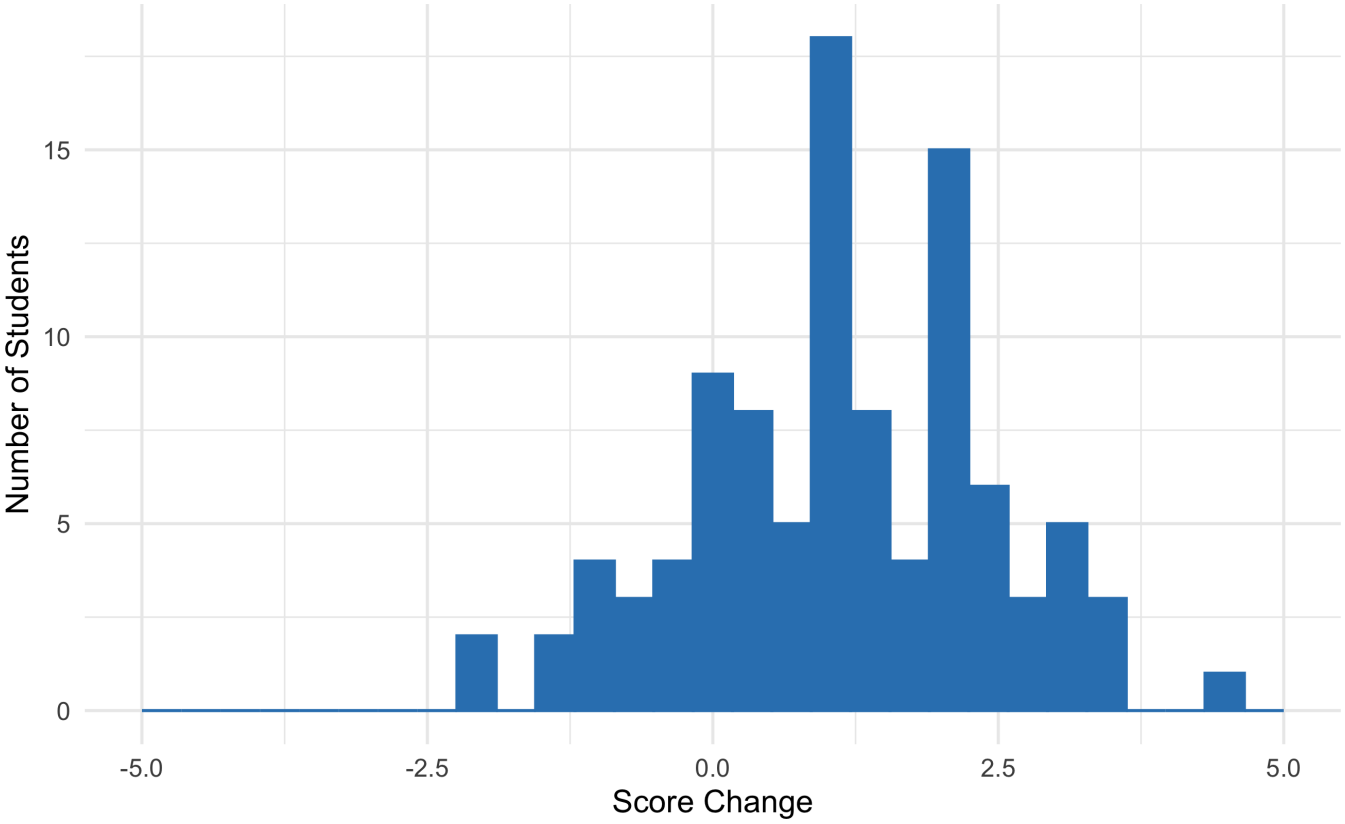

FIG 3 Student score changes on a low-stakes quiz after completing the lesson Most student scores improved between the pre- and post-lesson assessment. Score increases on the post-lesson assessment are shown as a positive change and score decreases on the post-lesson assessment are shown as a negative score change ($n = 100$).

Material S5. Supplemental Figures—Table S2. The greatest increase in confidence was observed for LO-2, suggesting that calculating surface-area-to-volume ratios of cells during the lesson helped students feel more confident about this skill.

In addition to evaluating student performance and confidence, we asked students to share reflections about their experiences using diagrams and models in the past and in this lesson. In the pre-lesson assessment, students were asked to reflect on models and images they had seen in past courses in an open-ended question (Supplemental Material S3. Lesson Assessment—SQ2). A prevalent theme in these responses was that students felt many images and models shown in biology courses are misleading about cell size or are not drawn to scale. Of the students who mentioned cell size or scale in their response, approximately 82% (42 of 51) of them indicated that cell size or scale was inaccurately represented or not specified in the models or diagrams they

TABLE 2 Frequency of correct responses to each question before and after completing the lesson[a]

Question	LO assessed	% of correct responses before lesson	% of correct responses after lesson	*p*
Q1	LO-2	45	62	0.004
Q2	LO-2	23	53	<0.001
Q3	LO-1	50	64	0.020
	LO-3			
Q4	LO-2	42	78	<0.001
Q5	LO-3	35	59	<0.001

[a]A McNemar test was used to evaluate the difference in frequencies of correct responses on the pre- and post-assessment for each question ($n = 100$). For Q5, students could earn partial credit. For each statement where they correctly checked or did not check the corresponding checkbox, they earned 0.2 points for a maximum of 1 point possible. For the purposes of the Q5 McNemar test, a response was considered correct when the student earned full credit. Partial credit was considered incorrect for these analyses.

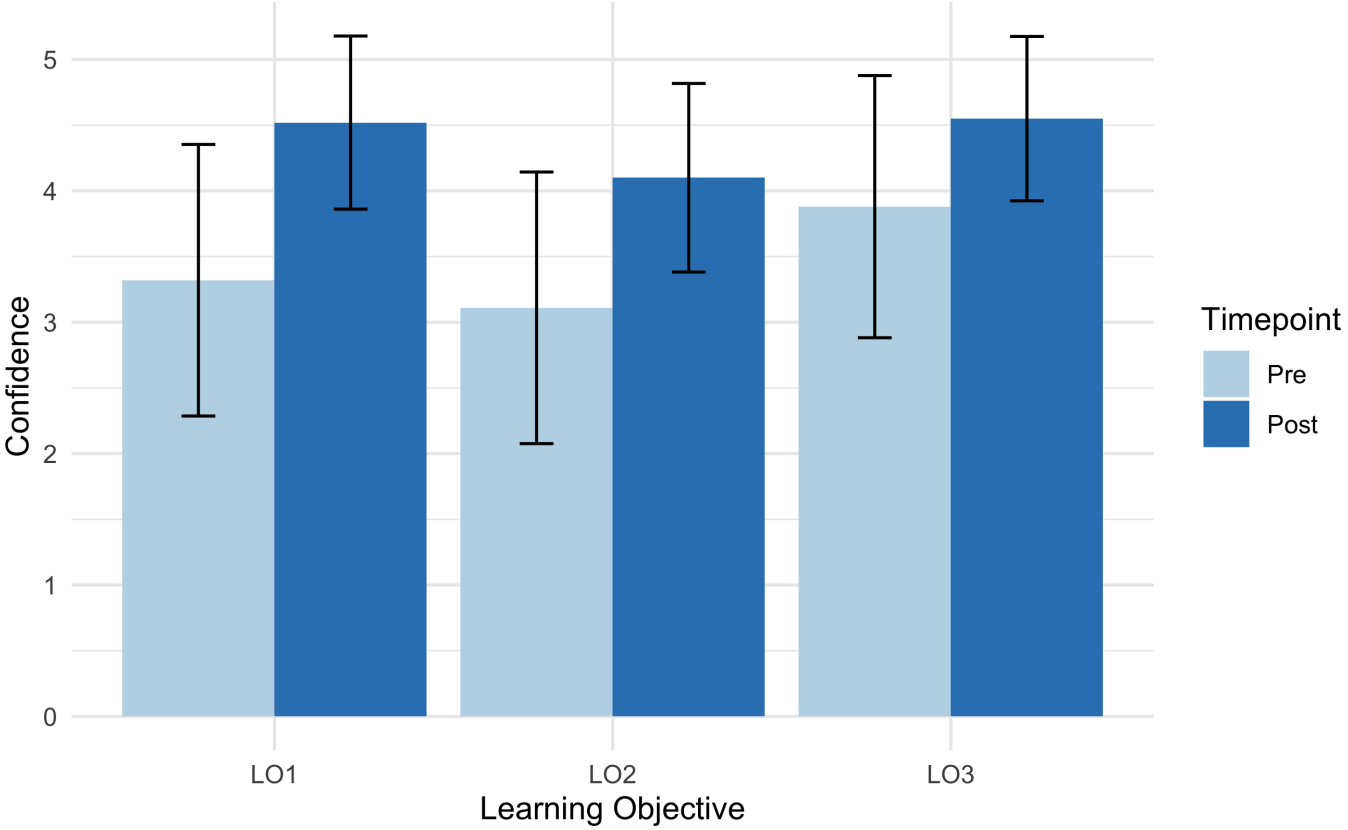

FIG 4 Student confidence in their knowledge of the learning objectives before and after completing the lesson Student confidence rating (5 = "very confident" through 1 = "not at all confident") on the pre- and post-lesson assessment improved for each learning objective. The error bars indicate one standard deviation of uncertainty. The observed increases in confidence were statistically significant based on a paired samples *t*-test for each of the three learning objectives (n = 100, $P < 0.001$). See Supplemental Material S5. Supplemental Figures—Table S2 for a statistical summary of the data.

had seen previously, indicating cell size and scale may often be overlooked in biology courses and textbooks. In their responses, several students mentioned that bacterial cells and eukaryotic cells are often implied to be similar sizes in side-by-side drawings. For example, one student wrote, "The models or drawings of cells that I have seen in other courses have often not accurately represented the relative sizes of real cells and their organelles. For example, often prokaryotic and eukaryotic cells will be compared side by side without any representation of their difference in size."

The models used in our lesson address this common misrepresentation, and students demonstrated increased knowledge about cell size by performing better on assessment questions about cell size after completing the lesson (Table 2). In addition, students felt more confident in their knowledge of the relative sizes of eukaryotic and bacterial cells (LO-2) and rated their confidence on average as 4.10 on a five-point scale (SD = 0.72), an increase of 0.99 points between the pre- and post-lesson timepoints [Fig. 4, $t(99) = 9.52$, $P < 0.001$].

Students were asked to share their thoughts about the models used in this lesson in an open-ended question on the post-lesson assessment (Supplemental Material S3. Lesson Assessment—SQ6). Many of the responses highlighted that the models filled a gap in their knowledge. For example, one student wrote, "I found the models to be extremely helpful, as previously I thought of all cells (eukaryotic and prokaryotic) as just small. It can be hard to conceptualize how different 'small' can be, but I think that scaling the cells up helped me to better understand."

Student responses were coded as positive, negative, or mixed as described in the "Suggestions for determining student learning" section. Overall, students responded

positively to the models, with 72.7% of the responses being coded as positive and 24.2% being coded as mixed (n = 99). These data suggest that the students perceived the 3D models as a positive aspect of this lesson, highlighting that 3D models may be a strategy worthy of further evaluation for its potential to increase student satisfaction with their biology coursework in other contexts.

Student perceptions of their learning were also positive. When asked to indicate the extent to which the lesson helped them learn concepts related to each of the learning objectives, most students rated the lesson either a four or five on a five-point scale (Supplemental Material S3. Lesson Assessment—SQ4), with average student ratings of 4.38 (SD = 0.80), 4.15 (SD = 0.94), and 4.37 (SD = 0.76) for LO-1, LO-2, and LO-3, respectively (Supplemental Material S5. Supplemental Figures—Fig. S3, n = 100). These findings were also supported by student responses to an open-ended question asking them to evaluate how the lesson helped them learn the concepts outlined in the learning objectives (Supplemental Material S3. Lesson Assessment—SQ6). Student responses indicated ways in which the lesson and models helped them understand all three of the learning objectives. Several of the responses specifically pointed out that the 3D models helped them better understand biological processes (LO-3), such as one student who wrote, "The imagery of the 3D printed models in relation to SA [surface area]:V [volume] ratio and efficiency of diffusion helped me understand the importance of SA:V ratio in biological processes. The [*T. namibiensis*] mechanism of compensating for a low SA:V ratio also helped me better understand this."

The results from our pre- and post-lesson assessment highlight that this lesson, including interacting with 3D cell models, promotes increased student knowledge of and confidence in the lesson learning objectives. In addition, students felt that the lesson helped them learn the concepts and generally reflected positively on their experience interacting with the 3D cell models. Together these data support that our lesson achieves its learning objectives and provides an effective strategy for teaching students about cell size and diffusion.

Possible modifications

This lesson could easily be adapted for use in an introductory biology course, non-majors course, or cell biology course. The models used in this lesson include several human cell types as well as bacteria and could be useful for various types of courses. The questions in the student worksheet focus on introductory microbiology concepts, but these questions could be modified to focus on concepts appropriate for students in alternative courses. For example, introductory biology students could be asked to consider the differences in the shapes of human osteoblasts and intestinal epithelial cells or to speculate about how the size of host-associated bacterial cells impacts their interactions with animal cells in the intestinal epithelial tissues. Where appropriate, students could also familiarize themselves with 3D modeling software by designing additional cell models themselves, which would present a unique multidisciplinary learning opportunity.

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The authors declare that they have no competing interests.

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ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental Material S1. Lesson Plan (jmbe00089-23-s0001.docx). Lesson plan outlining the timeline for the activity.
Supplemental Material S2. Lesson Worksheet (jmbe00089-23-s0002.docx). Worksheet to be provided to students.
Supplemental Material S3. Lesson Assessment (jmbe00089-23-s0003.docx). Assessment questions used to evaluate the lesson.
Supplemental Material S4. 3D Printing Instructions and Description of Models (jmbe00089-23-s0004.docx). Instructions and tips for 3D printing and additional information about the models.
Supplemental Material S5. Supplemental Figures (jmbe00089-23-s0005.docx). Supplemental figures and tables providing additional information about the analyses.

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